TABLE III.
Effect of Vacuum Drying on CO_2 Evolution by Zymin (Mg. CO_2 Evolved). Zymin 1 had been stored 0 days and Zymin 2 for 3 days.

	Zymin 1		Zymin 2	
Time (min.)	Control 45°	Vacuum Dried	Control 45°	Vacuum Dried
10	0.0	3.5	1.1	8.4
20	3.3	12.3	15.1	36.6
30	9.1	20.8	28.8	48.8
40	12.2	27.8	42.2	57.2
50	18.2	33.2	48.8	61.8
60	23.4	39.8	54.1	69.2
70	29.7	47.5	58.3	74.7
90	43.5	60.9	66.4	86.1

a more rapid decrease in rate after the maximum rate is reached. The maximum and steady rates are higher. The total evolution of carbon dioxide is 30-40% greater than in the control which had been dried at 45°. Parallel experiments also showed a greater rate of esterification of the inorganic phosphate in the presence of dextrose.

Summary. Storage of yeast at 5° varies the fermentative activity of zymin made therefrom. The activity increased up to 14 days' storage but the preparation from yeast which has been stored 20 days was inactive. Drying the preparation in vacuum, instead of the usual temperature of 45° markedly increases the activity of the zymin. The increase in activity with storage of the yeast or by employing vacuum drying is correlated with increased rate of esterification of the inorganic phosphate of the preparation in the presence of dextrose.

8424 C

Effect of Aeration and Lack of CO_2 on Growth of Bacteria-Free Cultures of Protozoa.*

THEODORE LOUIS JAHN. (Introduced by J. H. Bodine.)

From Iowa Lakeside Laboratory and Zoological Laboratory, State University of Iowa.

It has been known for some time that CO_2 is necessary for the growth of bacteria, yeast, and molds on solid media[1, 2] and recent

* Aided by a grant from the Rockefeller Foundation for work on the physiology of the normal cell.

1 Valley, G., and Rettger, L. F., *J. Bact.*, 1927, **14**, 101.

2 Valley, G., *Biol. Rev.*, 1928, **3**, 209.

investigations have shown that it is also necessary for bacteria when synthetic liquid media are used.[3, 4] In liquid media containing hydrolyzed protein growth is not completely inhibited, but it may be delayed considerably by the absence of CO_2.[4, 5] It has also been suggested that lack of sufficient CO_2 might be a cause of the lag period in growth of cultures of free-living protozoa and a cause of the failure of various investigators to obtain bacteria-free cultures of intestinal species.[6] For these reasons information concerning the effect of lack of CO_2 on protozoa seems highly desirable.

In the present experiments bacteria-free cultures of *Glaucoma piriformis*† and *Chilomonas paramecium*‡ were grown in a heavily buffered medium of 0.5% hydrolyzed casein and 0.25% KH_2PO_4 at pH 6.0 under the following conditions: (a) unaerated, in flasks with cotton stoppers, (b) aerated, in Drechsel gas washing bottles with ordinary air which had been washed through 2 bottles of water and an indicator solution (brom thymol blue), (c) aerated, in Drechsel bottles with CO_2-free air prepared by passing air through 2 Milligan spiral gas washing bottles of strong KOH and then through 2 bottles of water and an indicator solution. These conditions are similar to those used by Walker[5] for bacteria. The rate of bubbling was about 5 liters per hour per flask. The cultures were immersed in a stirred water bath, and the temperature was thereby maintained equal in all cultures and approximately that of the room (20°-25°C.). Each experiment was performed in duplicate and the results averaged. Counting methods used have been described previously.[7]

The experimental results are shown in Fig. 1. The ratio of the number of organisms present (x) at any time to the initial number (x_o) is plotted on a logarithmic scale with the value of log x/x_o marked on the left and the value of the ratio on the right ordinate; the abscissa is time in days. The data for Glaucoma represent a typical experiment. The aerated cultures grew more slowly during

[3] Walker, H. H., *Science*, 1932, **76**, 602; *J. Bact.*, 1932, **14**, 169.

[4] Gladstone, G. P., Fildes, P., and Richardson, G. M., *Brit. J. Exp. Path.*, 1935, **16**, 335.

[5] Winslow, C.-E. A., Walker, H. H., and Sutermeister, M., *J. Bact.*, 1932, **24**, 185.

[6] Jahn, T. L., *Cold Spring Harbor Symposia on Quant. Biol.*, 1934, **2**, 167.

† Strain isolated several years ago by Dr. Alford Hetherington of Stanford University.

‡ Strain isolated by present author and Dr. John B. Loefer of New York University in 1931.

[7] Jahn, T. L., *Biol. Bull.*, 1929, **57**, 81; 1931, **61**, 387.

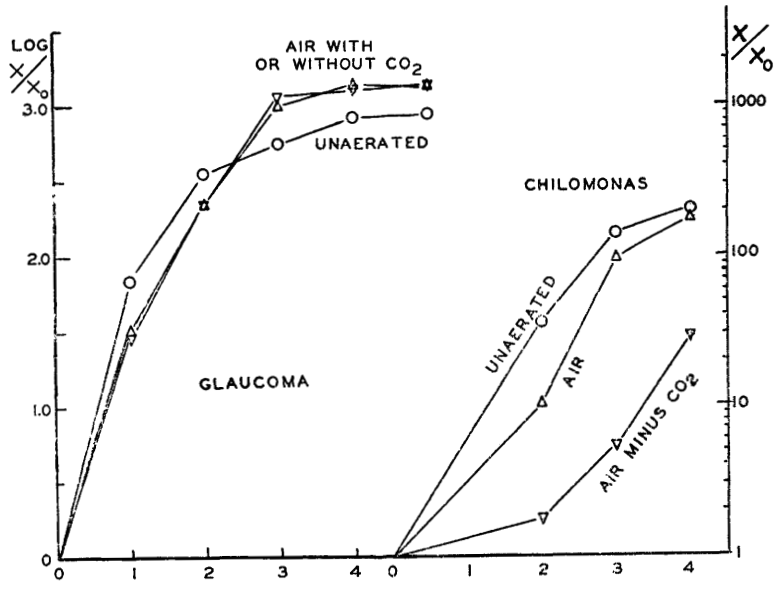

FIG. 1.

Graph showing the growth of cultures of Glaucoma and Chilomonas under different conditions. Ordinate: log x/x_0, where x_0 is the initial number and x is the number present at any given time. Value of the log is given on the left and value of the ratio on the right. Abscissa: time in days.

the first day, but the final number in these cultures was about 60% higher than that in the aerated cultures. It was found that after the second day the organisms in unaerated cultures were about 50% larger than during the first 2 days and that the protoplasm was high vacuolated. Those in the aerated cultures showed no gross morphological changes during the course of the experiment. The higher total number and lack of morphological changes in the aerated cultures are probably due to the maintenance of a constant gaseous content.§ There was no significant difference (*i. e.*, 15% or more) between the rate of growth in cultures aerated with ordinary air and those aerated with CO_2-free air. In a heavily buffered casein peptone medium, therefore, lack of CO_2 apparently had no effect on the growth of Glaucoma. Also, it is to be noted that no lag period was apparent, the rate of growth (slope of log x/x_0-time curve) being highest on the first day.

The data in Fig. 1 for Chilomonas represent the average values for 5 experiments, each of which was performed in duplicate. The unaerated cultures showed the highest initial growth rate and no

§ This does not occur in unaerated cultures of rapidly growing protozoa. (Jahn, T. L., *Arch. f. Protistenk.*, 1935, in press.)

apparent lag period. Those aerated with ordinary air showed a lower initial rate and a lag period. Those aerated with CO_2-free air showed the lowest initial growth rate and a more distinct lag period. No appreciable pH difference (greater than 0.1 unit) could be detected between cultures with or without CO_2. Lack of CO_2, therefore, causes a lower initial growth rate and a prolongation of the lag period in cultures of Chilomonas.

An explanation of why CO_2 should be necessary for growth of microorganisms has not been given in the literature. Gladstone, Fildes, and Richardson[4] considered several possibilities but offered no theory to explain the phenomenon. It seems quite evident from the literature that the optimum concentration of CO_2 for bacteria varies widely for the different species, and on the basis of available evidence this also seems to be true for protozoa.|| We might expect, therefore, a wide variation in the effect of almost complete removal of CO_2 such as has been found with bacteria and in the present experiments. Since cell membranes are freely permeable to CO_2 it seems a natural assumption that the bicarbonate buffering system in the protoplasm of cells which are adapted to living in a medium of high CO_2 content should be greater in magnitude and, therefore, relatively more important for the maintenance of the intracellular pH than that of cells which ordinarily do not live in high CO_2 tension. If all of the CO_2 is removed from the medium, CO_2 will also be drawn from the protoplasm, thereby causing a shift in the bicarbonate buffer system and possibly an appreciable change in pH. If the bicarbonate system is of minor importance this change might be negligible, but if bicarbonate is the principal buffer involved the change would be of considerable importance. It seems, therefore, that this might be the key to an explanation of the necessity of CO_2. The effectiveness of the removal of CO_2 from the medium in removing CO_2 from the cell will depend, of course, on the rate of production of CO_2 by the organism which will vary with different media and with O_2 tension.[4]

On this theory one should expect CO_2 to be replaceable to some extent by other weak acids which are partly dissociated at the pH of the medium. The ionic fraction of such acids might be inhibited electrically from passing through the membrane, whereas phosphoric acid at the pH of the medium is completely in the form of ions, and

|| See Jacobs, M. H., (*J. Exp. Zool.*, 1912, **12**, 519) for differences in higher limits and present data for lower limits of CO_2 effect. Chilomonas is found in greatest numbers in very putrid cultures which undoubtedly have a high CO_2 content.

therefore, probably highly impermeable. Certain amino acids, however, are partially undissociated and probably permeable and therefore should decrease or prevent the deleterious effects of CO_2-free media. The necessary concentration of the substitute acid should vary with the CO_2 tension otherwise necessary for the species, and since the permeability of the cell to amino acids is probably considerably lower than to CO_2 complete substitution might not be possible in some cases. This fits the experimental evidence in that amino acids may partially replace CO_2 for growth of certain bacteria but that they seem ineffective for others[4] (anaerobes or anaerobic conditions). In all aerobic conditions tried, however, CO_2 can be partially replaced by hydrolyzed protein.[3, 4, 5] This might be due to a higher concentration of effective acids and/or to the higher production of CO_2 by the bacteria in these media. This theory seems to explain satisfactorily all the known data on the lack of CO_2 on microorganisms. Final proof, however, will involve measurements of the intracellular pH under various CO_2 tensions.